REMARKS

With entry of this amendment, the status of the claims is as follows:

Original:	6, 10, 12-15, 25 and 32
Currently amended:	1 and 4
Previously presented:	3, 5, 7-9, 11, 21-24, 27, 31 and 37
Canceled:	2, 16-20, 26, 28-30 and 33-36

With entry of this amendment, claims 1, 3-15, 21-25, 27, 31, 32 and 37 are pending. Entry of the amendment is requested, because it is believed the amendment places the application in condition for allowance by canceling certain claims and making minor revisions to other claims in order to overcome section 112 rejections. The particulars of the claim changes related to the rejections under 35 U.S.C. § 112 are described in the remarks which follow.

Previously withdrawn claims 16-20, 28, 29 and 33-36 have also been canceled herein.

Applicants reserve the right to pursue claims directed to any of the canceled subject matter in one or more continuing applications.

Interview Summary

The undersigned and the Examiner participated in a telephonic interview on October 21, 2004 (hereinafter referred to as the "interview"). The first issue discussed was the rejection of claims 26 and 30 under 35 U.S.C. § 112, first paragraph, as not being enabled by the description. Just prior to the interview, the undersigned faxed to the Examiner a copy of Hazuda et al., "Inhibitors of Strand Transfer That Prevent Integration and Inhibit HIV-1 Replication in Cells", *Science* 2000, 287: pp. 646-650, a copy of which is enclosed herewith as well. The undersigned presented the following argument in support of the enablement of claims 26 and 30:

> Hazuda et al. note that integration involves three steps: (i) assembly of a stable preintegration complex, (ii) 3' processing, and (iii) strand transfer, wherein assembly is a prerequisite for catalysis. Hazuda et al. further note that some compounds may appear to inhibit 3' processing and strand transfer but have no effect on either step when assayed subsequent to assembly; i.e., the compounds are not true integrase inhibitors. In order to identify inhibitors of integrase catalysis, Hazuda et al. disclose that they "biased the strand transfer reaction by means of preassembling recombinant integrase on immobilized oligonucleotides as a surrogate for preintegration complexes." (see p. 646 of Hazuda et al.) Using this strand transfer assay Hazuda et al. identified a series of diketo acid compounds that inhibit the strand transfer reaction and for the most potent analogs inhibit HIV-1 replication in cell culture. Hazuda et al. then employed two of the more active diketo acids in several experiments validating integrase as the molecular target. For example, Hazuda et al. found that variants of wild type HIV-1 resistant to the diketo

acids contained mutations in the integrase coding region of their cDNA (see p. 647). Hazuda et al. also determined that the diketo acids affect integration activity without affecting synthesis or 3' processing of the viral DNA (see p. 648). Hazuda et al. also found that in cells acutely infected with wild-type HIV-1, the presence of a diketo acid resulted in the accumulation of integration-incompetent circular viral DNA products and a decrease of integration-competent linear viral DNA products (see p. 649).

The person of ordinary skill in the art would understand from reading Hazuda et al. that substances exhibiting inhibition activity in the assay disclosed therein (i.e., a strand transfer assay using preassembled integrase strand transfer complexes) are HIV integrase inhibitors. The assay employed to measure the integrase inhibition activity of the instantly claimed compounds (i.e., the assay described in Example 193 of the subject application) is a strand transfer assay using preassembled complexes of the type described by Hazuda et al. Accordingly, the person of ordinary skill in the art reading Example 193 in view of Hazuda et al. would not find the utility recited in claims 26 and 30 (i.e., integrase inhibition by the claimed compounds) to be unusual or speculative.

Further support of the enablement of claims 26 and 30 can be found in the Hazuda Declaration, which was submitted with the amendment filed May 6, 2004. The Hazuda Declaration presents evidence confirming that the claimed compounds are integrase inhibitors. The Examiner has given little or no weight to the evidence in the Declaration, because it is necessary that the claimed invention be enabled as of the time of filing and the Declaration was submitted subsequent to the filing date. However, while it is necessary to demonstrate that the disclosure as filed enables the claimed invention, this (per MPEP § 2164.05, p. 2100-190) "does not preclude applicant from providing a declaration after the filing date which demonstrates that the claimed invention works." The Hazuda Declaration is a declaration demonstrating that the claimed invention works; i.e., that the claimed compounds are in fact integrase inhibitors. The experimental methods employed therein were known to those of ordinary skill in the art prior to the filing date of the subject application. Indeed some of the methods are essentially the same as those reported in the Hazuda et al. *Science* 2000 article cited above; see, for example, the results for L-870,810 set forth in Paragraphs 9 and 10 of the Hazuda Declaration.

In summary, claims 26 and 30 are enabled as of the filing date, because the subject application discloses that compounds representative of the invention are integrase inhibitors (see Example 193) and, in view of the Hazuda et al. *Science* article, the person of ordinary skill in the art would not question this utility. Furthermore, the skilled artisan at the time the application was filed could have confirmed the utility without undue experimentation in the manner set forth in the Hazuda Declaration.

In view of the foregoing, the section 112 rejection should be withdrawn.

The Examiner requested some time to consider these arguments and to consult with others in the Patent Office. She telephoned the undersigned later in the day of the interview to say that her position was that the claims were enabled with respect to the specific compounds of the invention that exhibited activity in the assay set forth in Example 193, but were not enabled for the compound genus. Applicants' position is that the compounds actually tested in the assay are representative of compounds embraced by the genus and thus claims 26 and 30 are fully enabled. In any event, the Examiner maintained the rejection.

The second issue discussed in the interview was the rejection of claims 1, 3-15, 25-27 and 32 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement because the term "$N(H)R^t$" in the definition of R^k in claim 1 constitutes new matter. The undersigned asked the Examiner to reconsider the rejection employing essentially the same argument presented in the Response after Final Rejection filed August 16, 2004:

> Claim 4 as originally defined depends from claim 1 and accordingly incorporates by reference all the limitations set forth in claim 1. In original claim 4 the definition of R^k includes a saturated heterocyclic ring which is optionally substituted with, *inter alia*, $N(R^a)R^t$, wherein R^a is H or C_{1-4} alkyl. This is inconsistent with the definition of R^k in original claim 1, which recites "N-heteromonocyclyl-N-C1-6 alkyl-amino-". The person of ordinary skill in the art would understand this inconsistency to be due to the inadvertent omission of the appropriate antecedent in claim 1 and thus would recognize that the Applicants were in possession of compounds of Formula I in claim 1 in which the definition of R^k includes the substituent $N(H)R^t$.

The Examiner remained unpersuaded by this argument. Her position was that introduction of the term $N(H)R^t$ in claim 1 unreasonably broadens the scope of claim 1 compared to claim 1 as originally filed and thereby introduces new matter. Accordingly, no agreement was reached and the rejection was maintained. The Examiner indicated, however, that she would consider withdrawing the rejection, if the term $N(H)R^t$ were removed from the definition of R^k in claim 1 and claim 4 was rewritten in independent form.

The third and final issue discussed in the interview was the rejection of claim 11 under under 35 U.S.C. § 112, second paragraph, as being indefinite due to a lack of antecedent basis. The undersigned argued that the rejection should be withdrawn, because claim 11 was an independent claim. The Examiner agreed to withdraw the rejection.

<u>First Rejection under 35 U.S.C. § 112, first paragraph</u>

Claims 26 and 30 have been rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the description. The undersigned presented arguments in the telephonic interview (see summary above) that claims 26 and 30 are enabled, but the Examiner was not fully persuaded by these arguments and indicated she would maintain the rejection. In order to

expedite prosecution of this application, claims 26 and 30 have been canceled herein, rendering this rejection moot. Withdrawal of the rejection is accordingly requested.

Second Rejection under 35 U.S.C. § 112, first paragraph

Claims 1, 3-15, 25-27 and 32 have been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner has asserted that the definition of the substituent $N(R^a)R^t$ for R^k constitutes new matter when R^a is hydrogen or C_{1-6} haloalkyl. The undersigned discussed this rejection with the Examiner during the telephonic interview (see summary above). As a result of that discussion, claim 1 has been amended to replace "$-N(H)R^t$ or $-N(C_{1-6}$ alkyl)R^t" in the definition of R^k with "$-N(C_{1-6}$ alkyl)R^t", and claim 4 has been rewritten in independent form. Claim 1 as originally filed provides clear support for $-N(C_{1-6}$ alkyl)R^t. The claims as amended do not contain new matter and do comply with the written description requirement. Withdrawal of this section 112 rejection is accordingly requested.

Rejection under 35 U.S.C. § 112, second paragraph

Claim 11 has been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner has asserted a lack of antecedent basis for $-N(R^a)-C(=O)-CH_2)_{1-2}-C(=O)-N(R^a)_2$ in the definition of R^k. As a result of the telephonic interview (summarized above), the Examiner indicated that the rejection would be withdrawn.

Provisional Obviousness-type Double Patenting Rejections

Claims 1, 3-15, 21-27, 30-32 and 37 have been provisionally rejected for obviousness-type double patenting over claims in each of the following copending applications:

A) U.S. Application No. 10/399,083 (Attorney Docket No. 20758YP).
B) U.S. Application No. 10/486,535 (Attorney Docket No. 20950Y).
C) U.S. Application No. 10/398,929 (Attorney Docket No. 20760YP).
D) U.S. Application No. 10/218,537 (Attorney Docket No. 20951Y).

In view of the earlier-described amendments to the claims and the remarks on the section 112 rejections, it is believed that the application is in condition for allowance apart from the provisional double patenting rejections A to D. In accordance with paragraph I.B of MPEP § 804, it is requested that the provisional rejections be withdrawn in this application and that the application be permitted to issue. The Examiner is asked to telephone the undersigned should any minor matters need to be resolved before a Notice of Allowance can be mailed.

Respectfully submitted,

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8. Increased MR responses to repeated distracters were found in only two participants. In both participants, these increased responses were in the intraparietal sulcus.
9. Analysis of the MR responses to nonrepeated distracters at each of the 13 stimulus positions within a trial did not show the same trend as the targets or distracters. Thus, we discount the possibility that the repetition reduction effect reflects a "position effect" or linear trend within a trial. Similar results were found for the intraparietal area responses.
10. MR responses to repeated distracters reset in subsequent trials to initial levels in both left and right ventral temporal areas (Fig. 4B presents the mean over two hemispheres) as well as in the left and right intraparietal areas. In all cases, the response to the first presentation in later trials was not significantly different from the response to the first presentation in first trials ($P > 0.1$).
11. R. Buckner *et al.*, *J. Neurosci.* **15**, 12 (1995); J. Demb *et al.*, *J. Neurosci.* **15**, 5870 (1995); D. Schacter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 321 (1996); L. Squire *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1837 (1992); C. Büchel, J. T. Coull, K. J. Friston, *Science* **283**, 1538 (1999).
12. Six participants performed the same working memory task as in the fMRI study, except that they responded to both targets and distracters. The median RT for repeated distracters (429 ms) was significantly shorter than that for nonrepeated distracters (491 ms): $F(1,5) = 6.7$, $P < 0.05$. RTs for repeated distracters were computed separately for each of five repetitions. A significant main effect of repetition, $F(4,20) = 7.2$, $P < 0.001$, indicated that RT declined with repeated presentation (from 447 to 396 ms). Thus, repetition of familiar objects during the working memory task was associated with improved performance in detecting distracters.
13. E. K. Miller and R. Desimone, *Science* **263**, 520 (1994); E. K. Miller, C. Erickson, R. Desimone, *J. Neurosci.* **16**, 5154 (1996).
14. L. Li and R. Desimone, *J. Neurosci.* **13**, 1460 (1993); W. Suzuki, *Neuron* **24**, 295 (1999).
15. R. Parasuraman, in *The Attentive Brain*, R. Parasuraman, Ed. (MIT Press, Cambridge, MA, 1998), pp. 3–15.
16. R. Desimone and J. Duncan, *Annu. Rev. Neurosci.* **18**, 193 (1995).
17. We thank R. Desimone for insightful comments on an earlier version of the manuscript, J. Maisog for implementing data analysis software, J. Schouten and E. Hoffman for participant recruitment and training, L. Kikuchi and C. Chavez for conducting the behavioral study, J. Szczepanik for help with data analysis, S. Courtney and L. Petit for valuable discussions, and the NIH in vivo Nuclear Magnetic Resonance Center for assistance with MR imaging. Y.J. and R.P. were supported by NIH grant AG07569.

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Inhibitors of Strand Transfer That Prevent Integration and Inhibit HIV-1 Replication in Cells

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Integrase is essential for human immunodeficiency virus–type 1 (HIV-1) replication; however, potent inhibition of the isolated enzyme in biochemical assays has not readily translated into antiviral activity in a manner consistent with inhibition of integration. In this report, we describe diketo acid inhibitors of HIV-1 integrase that manifest antiviral activity as a consequence of their effect on integration. The antiviral activity of these compounds is due exclusively to inhibition of one of the two catalytic functions of integrase, strand transfer.

The development of chemotherapeutic agents for the treatment of HIV-1 infection has focused primarily on two viral enzymes: reverse transcriptase and protease. Although regimens including agents directed at each of these biochemical targets are effective in reducing viral load and morbidity and mortality, the long-lived nature of the infection and the genetic plasticity of the virus have made it apparent that new antiretroviral agents are required to deal with the appearance and spread of resistance (*1*). HIV-1 integrase catalyzes the insertion of the viral DNA into the genome of the host cell. Integration is essential for viral replication and is thus an attractive target for novel chemotherapy (*2, 3*). Many inhibitors of HIV-1 integrase have been identified; however, their in vitro activity has not translated into antiviral activity in cells (*4*).

Integration is a multistep process that occurs in discrete biochemical stages: (i) assembly of a stable complex with specific DNA sequences at the end of the HIV-1 long terminal repeat (LTR) regions, (ii) endonucleolytic processing of the viral DNA to remove the terminal dinucleotide from each 3′ end, and (iii) strand transfer in which the viral DNA 3′ ends are covalently linked to the cellular (target) DNA (Fig. 1) (*4*). Each of the catalytic reactions (3′ processing and strand transfer) requires integrase to be appropriately assembled on a specific viral DNA (or donor) substrate (*5*). In general, compounds identified in assays with purified, recombinant integrase interfere with assembly in vitro (*6, 7*). Because assembly is a prerequisite for catalysis, such compounds may appear to inhibit 3′ processing and strand transfer, but they have no effect on either reaction when assayed subsequent to assembly on HIV-1–specific oligonucleotides (*6*). These compounds are also ineffective in assays wherein viral preintegration complexes isolated from HIV-1–infected cells are used (*8*).

To identify inhibitors of catalysis, we biased the strand transfer reaction by means of preassembling recombinant integrase on immobilized oligonucleotides as a surrogate for preintegration complexes (*6*) (Fig. 1). In a random screen of more than 250,000 samples, a variety of inhibitors was identified; however, the most potent and specific compounds each contained a distinct diketo acid moiety, and thus these inhibitors segregate into a single structural class (Fig. 1). The diketo acid functionality is an intrinsic feature of these inhibitors but is not sufficient for activity, as structural analogs exhibit a range of inhibitory potency. For most analogs, the activity observed in strand transfer assays with recombinant integrase correlated with their relative activity in assays using HIV-1 preintegration complexes (*9*). Analogs that were more potent in these biochemical assays also inhibited HIV-1 replication in cell culture.

L-731,988 and L-708,906 were two of the most active diketo acids in strand transfer assays with recombinant integrase. With 50% inhibitory concentrations (IC_{50}'s) of 80 and 150 nM, respectively, L-731,988 and L-708,906 are also the most potent inhibitors of preintegration complexes described to date. In a single-cycle assay for acute infection (*10*), L-731,988 and L-708,906 inhibited HIV-1 replication with IC_{50}'s of 1 to 2 μM; higher concentrations prevented the spread of HIV-1 in cell culture for several weeks (Fig. 2). L-731,988 and L-708,906 were comparably active against both macrophage- and T cell line–tropic strains of HIV-1, clinical isolates, and variants resistant to reverse transcriptase and protease inhibitors (*11*). Consistent with the effect of an early stage inhibitor, the compounds did not affect virus production from persistently infected cells (up to 50 μM) (*11*).

To validate integrase as the molecular target responsible for the antiviral effect, we selected HIV-1 variants resistant to L-708,906 and L-731,988. At concentrations of inhibitor sufficient to block replication of the wild-type virus (20 μM), the resistant variants replicated nearly as well as the wild-type (or resistant) virus in the absence of inhibitor (Fig. 2). Sequencing of the cDNA derived from four resistant populations consistently identified specific mutations

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within the integrase coding region (Table 1). $Met^{154} \rightarrow Ile^{154}$ (M154I) was detected in 18 clones from three populations selected independently for L-731,988 resistance. Although the L-708,906 population was more heterogeneous, M154I was identified in a subset of these variants. $Ser^{153} \rightarrow Tyr^{153}$ (S153Y) and $Thr^{66} \rightarrow Ile^{66}$ (T66I) were also routinely observed in the L-708,906–resistant population; T66I was identified in all but one sequence and was nearly always accompanied by either S153Y or M154I. Other changes in integrase were detected; however, only T66I, S153Y, and M154I were observed consistently, and every clone contained one or more of these mutations. The observation that Thr^{66}, Ser^{153}, and Met^{154} are conserved and proximal to the active site residues Asp^{64} and Glu^{152} (*12*) suggests that these inhibitors bind at or near the enzyme active site. No changes were detected in either the U5 or U3 LTR.

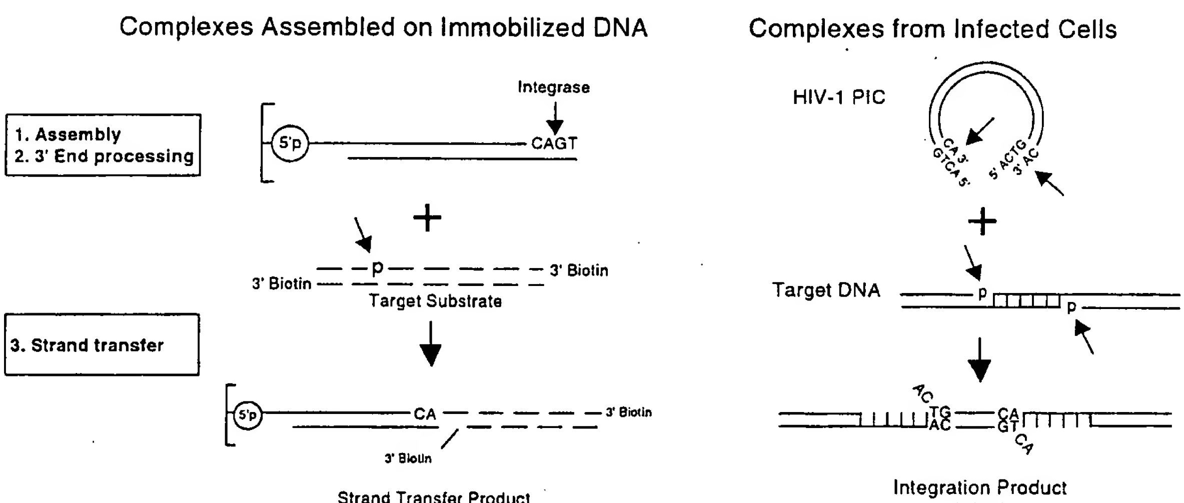
To demonstrate that the mutations observed in integrase were necessary and sufficient for resistance, we engineered each of the substitutions into an otherwise isogenic recombinant virus and into the recombinant integrase protein and evaluated susceptibility to L-708,906 and L-731,988. Each mutation conferred some degree of resistance to one or both inhibitors (Table 1). M154I engendered modest resistance (two- to fivefold) to both compounds, whereas S153Y affected the relative susceptibility to L-708,906 but was not sufficient to confer resistance to L-731,988. These results are consistent with the observation that only M154I was present in the L-731,988–resistant population. The data also suggest that the two inhibitors differ subtly in their interaction with the enzyme.

At best, individual mutations in integrase conferred a six- to sevenfold loss of susceptibility to either L-708,906 or L-731,988; however, increased resistance was achieved when the mutations were combined pairwise, as observed in the L-708,906–selected population. In a single-cycle HIV-1 infection assay (*10*), the combination of T66I and S153Y produced the largest effect with the IC_{50} for L-708,906 shifted by more than 20-fold, as compared with two- to threefold for either mutation alone (Table 1). The mutant viruses remained sensitive to inhibitors of protease and reverse transcriptase, e.g., L-697,661 (Table 1) (*13*). Although individual mutations did not appear to impair catalytic activity or infectivity, double mutants displayed decreased enzymatic activity and

Table 1. Mutations in HIV-1 integrase confer resistance to diketo acid inhibitors. ND, not determined; Und., undetermined.

Constructed mutations	Strand transfer (IC_{50}, μM)		HIV-1 infectivity (IC_{50}, μM)		
	L-708,906	L-731,988	L-708,906	L-731,988	L-697,661*
WT HXB2	0.1	0.1	2.5	1.0	0.03
T66I	0.6	0.6	8.0	7.0	ND
S153Y	0.6	0.2	10	1.0	ND
M154I	0.5	0.5	5.0	4.0	ND
T66I/S153Y	Und.†	Und.†	>50	10.0	0.04
T66I/M154I	1.0	1.5	16.0	12.0	0.05

*Nonnucleoside inhibitor of HIV-1 reverse transcriptase (*13*). †Activity against the T66I/S153Y mutant could not be determined because of poor activity of the purified protein.



Compound	Strand Tranfer (recombinant Integrase)	HIV-1 Preintegration Complexes	HIV-1 Infectivity
L-731,988	0.05	0.08	1.0
L-708,906	0.10	0.15	2.0
L-731,927	0.50	0.40	15
L-731,942	7.50	>100	>50

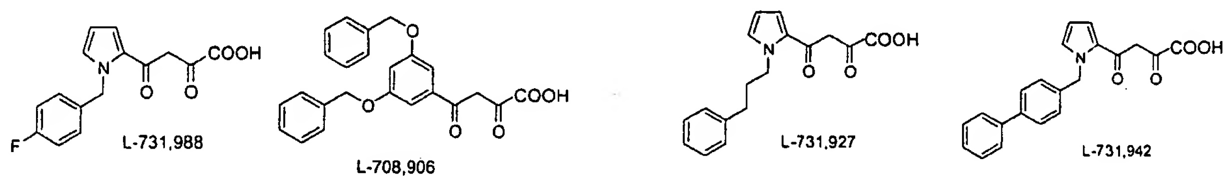


Fig. 1. Activity (IC_{50}, in μM) of diketo acid inhibitors. PIC, preintegration complex.

altered replication kinetics (14). The T66I/S153Y enzyme exhibited less than 5% of the activity of wild-type integrase; therefore, IC_{50}'s could not be determined.

To define the mechanism by which these compounds inhibit integrase and HIV-1 replication, we evaluated the activity of L-731,988 in relation to each of the discrete steps required for integration. In biochemical assays, the concentrations of L-731,988 required to inhibit 3′ processing were 70 times higher than the concentrations required to inhibit strand transfer (6 μM versus 80 nM, respectively) (Fig. 3). Disparate potency was also observed for disintegration (15), where the IC_{50} for L-731,988 was ~20 μM with either the core domain (amino acids 50 through 212) or the full-length enzyme (16).

The concentration of L-731,988 required to inhibit 3′ processing (or disintegration) was greater than that required to inhibit acute infection (6 μM versus 1 μM). Therefore, to assess the effect on integrase activity in the context of HIV-1 replication, we analyzed preintegration complexes extracted at various times after infection. The kinetics of reverse transcription and 3′ processing of the U5 LTR (and U3 LTR) by integrase were identical in cells infected in the presence or absence of L-731,988. (Fig. 4A) (17). In contrast, preintegration complexes isolated from cells infected in the presence of L-731,988 were not competent to integrate when tested in vitro (Fig. 4B). The observation that L-731,988 and related inhibitors (e.g., L-708,906) affect integration activity without affecting synthesis or processing of the viral DNA is consistent with the selective profile that the compounds manifest toward strand transfer in biochemical assays. L-731,988 inhibits the final catalytic step in integration, and this specific effect on integrase is sufficient to account for the antiviral properties of the inhibitor.

To assess the outcome of this effect in infected cells, we investigated the nature of the HIV-1 DNA produced as a consequence of L-731,988 inhibition. Cells infected with wild-type HIV-1 contain both linear and cir-

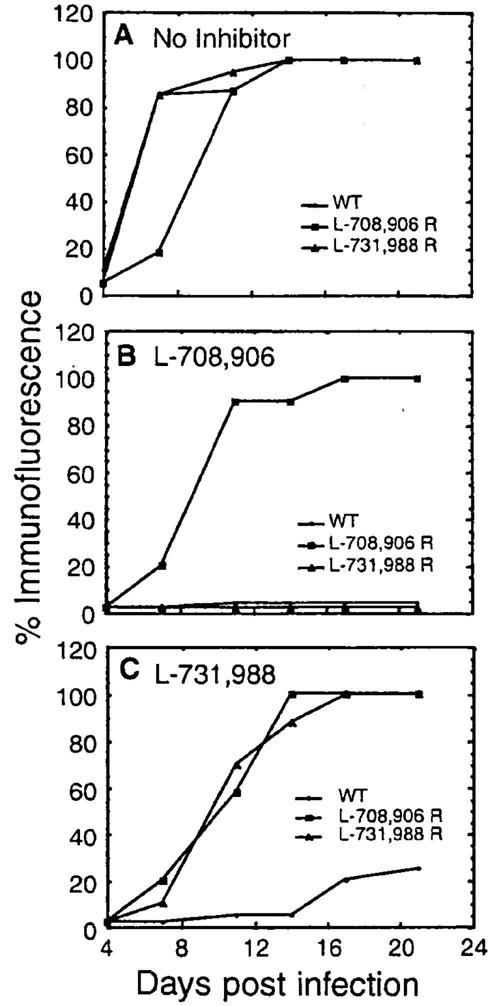


Fig. 2. Effect of L-708,906 and L-731,988 on the replication of wild-type (WT) HIV-1 and diketo acid–resistant variants. Viral replication in H9 T-lymphoid cells was assayed by immunofluorescent staining for HIV-1–specific antigens (19). Cultures were incubated (A) in the absence of inhibitor or in the presence of (B) 20 μM L-708,906 or (C) 20 μM L-731,988. For each condition, the replication of wild-type HIV-1 IIIB and virus populations selected for resistance to L-708,906 and L-731,988 (20) was evaluated.

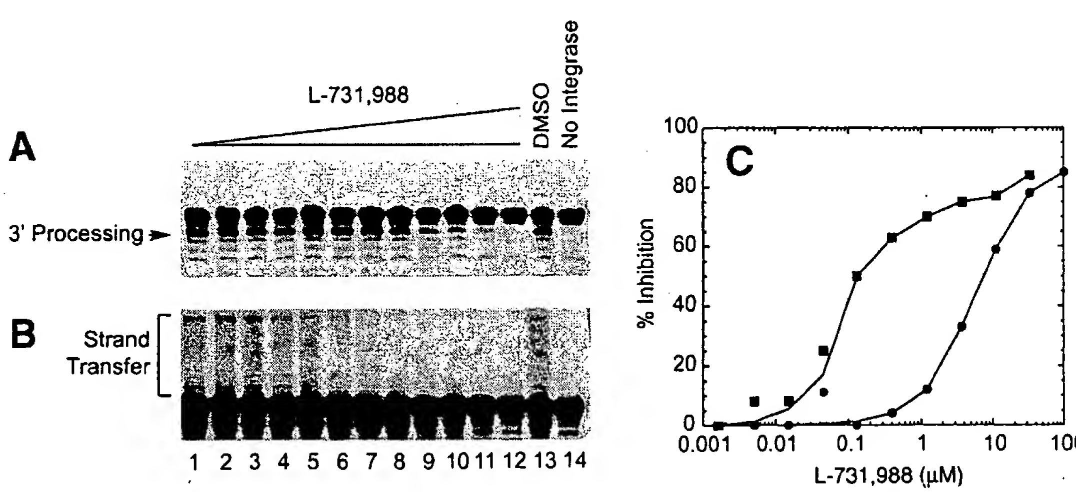
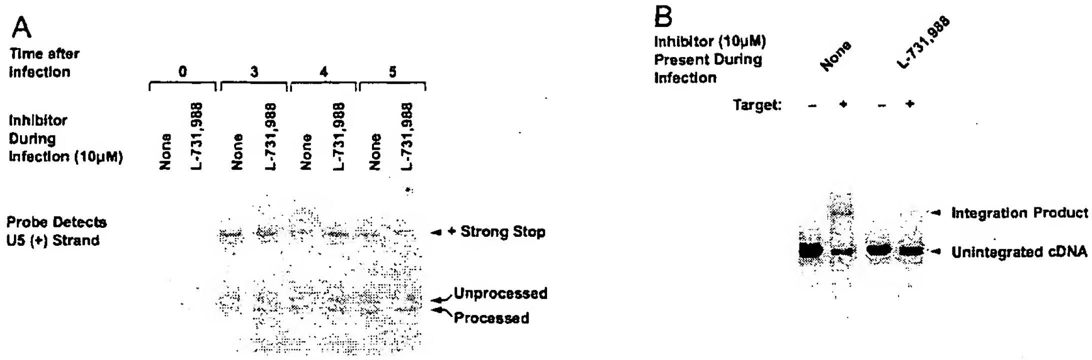


Fig. 3. Inhibition of 3′ processing and strand transfer by L-731,988. (A) Integrase 3′ processing and (B) strand transfer activities are shown. HIV-1 HXB-2 integrase (1 μM) was incubated with 50 nM of a ^{32}P-labeled oligonucleotide representing the last 20 nucleotides of the HIV-1 U5 end. Reactions included varying levels of L-731,988 (threefold dilutions from 100 μM, lane 12, to 5 × 10^{-4} μM, lane 1). Reactions were performed for 1 hour at 37°C and analyzed as described (18). The fraction of substrate converted to the specific 3′ processed product (18-nucleotide oligomer) or to strand transfer products (>20 nucleotides) was determined by PhosphorImager analysis. (C) Percent inhibition in relation to the DMSO control (lane 13); the IC_{50}'s determined for inhibition of 3′ processing (circles) and strand transfer (squares) (6 and 0.08 μM, respectively) are representative of multiple experimental determinations.



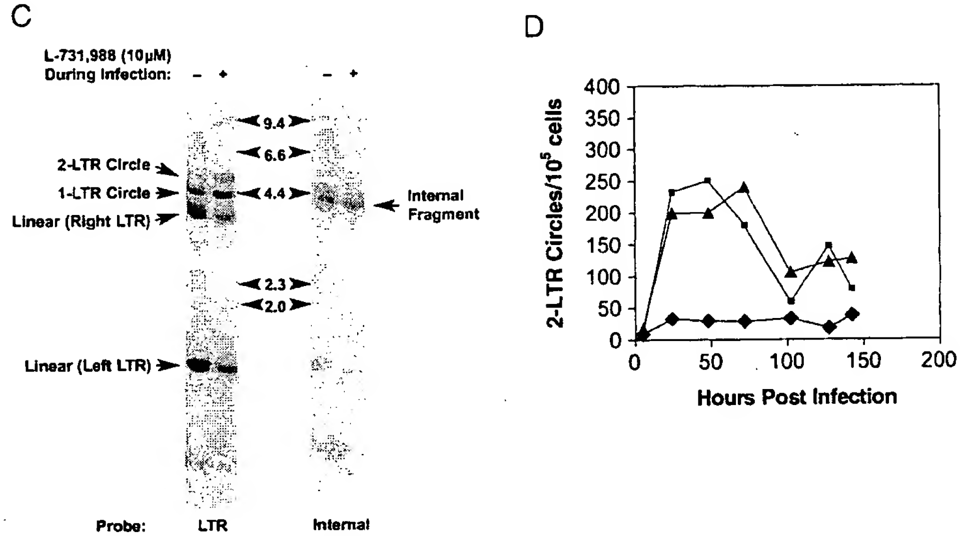


Fig. 4. Effects of L-731,988 on HIV-1 DNA in acutely infected cells. (A) Kinetics of reverse transcription and 3′ processing. Molt IIIB/Sup T1 cocultures were established, and cytoplasmic extracts were made at various times after infection (*8*, *21*). Where appropriate, Sup T1 cells were preincubated with L-731,988 or L-708,906 for 1 hour before coculture. Analysis of the U5 3′ DNA end was done by indirect end-labeling of restriction digest fragments (*21*). Positions of the unprocessed (blunt) U5 ends, the 3′ processed U5 ends, and the plus strand strong-stop DNA (reverse transcription intermediate) are indicated. (B) In vitro activity of preintegration complexes from cells infected for 5 hours in the presence or absence of L-731,988. Preintegration complexes were isolated [as in (A)] and then incubated with (+) or without (−) φX174 target DNA; reactions were analyzed by Southern blotting (*22*). (C) HIV-1 DNA forms in infected cells in the presence or absence of L-731,988. After coculture for 24 hours [as in (A)], low-molecular-weight DNA was prepared. The DNA was digested with Sal I and Pst I and analyzed by Southern blotting with an HIV-1 LTR probe to detect all products or an internal probe. (*21*). Positions of linear and circular HIV-1 DNA forms are indicated. (D) Sup T1 cells were infected with wild-type and integrase-defective NL4-3 (*18*). DNAs were prepared and analyzed by Southern blotting [as in (C)] and by a quantitative TaqMan assay for HIV-1 two-LTR circular DNA. Diamonds, wild-type NL4-3 control; squares, wild-type NL4-3 with 10 μM L-731,988; and triangles, NL4-3 with the integrase D116N mutation (*18*).

cular viral DNA products (*3*). Circular forms of HIV-1 DNA are the products of cellular enzymes in the nucleus and are not competent for integration. With integrase-deficient viruses, circular DNA forms accumulate (*3*). In cells acutely infected with wild-type HIV-1, the presence of L-731,988 also led to an accumulation of circular products and a concomitant decrease in integration-competent linear viral DNA (Fig. 4C). The effect of L-731,988 on HIV-1 infection was indistinguishable from an integration-defective mutation [integrase $Asp^{116} \rightarrow Asn^{116}$ (D116N)] (*18*); in both infections, two LTR circles peaked within 24 hours at a level ~10 times the level observed in the absence of inhibitor (Fig. 4D). Similar results were obtained for L-708,906 and other active diketo acids (*17*). By altering the kinetics of the strand transfer, the diketo acids bias the formation of replication-defective products and inhibit HIV-1 replication.

We have presented L-731,988 and the diketo acids as the archetype of a new class of integrase inhibitors and novel antiretroviral agents. The compounds are specific inhibitors of integration, which exert their antiviral effect on HIV-1 solely as a consequence of their ability to inhibit the strand transfer activity of integrase. The compounds exhibit a preference for the strand transfer reaction in vitro and inhibit integration without affecting synthesis or processing of the HIV-1 DNA in infected cells. Mutations proximal to the integrase active site residues engender resistance to the virus and the isolated enzyme, establishing the diketo acids as the first biologically validated inhibitors of integration. Although the same active site residues are required for 3′ processing and strand transfer (*12*), the ability of the diketo acids to discriminate between the two catalytic functions of integrase implies that a unique conformation of the enzyme may mediate strand transfer. The association of integrase with LTR sequences results in the formation of a stable, active strand transfer complex (*5*). We have noted that HIV-1 LTR sequences stimulate high-affinity binding of L-731,988 and related compounds to integrase, suggesting that integrase may adopt a distinct conformation subsequent to assembly (*16*). Together with the results presented, these observations provide biochemical and physical evidence dissociating the two catalytic functions of integrase and suggest that the diketo acids may be useful tools to probe the enzymatically active structure of integrase and to investigate the complexities of this reaction.

References and Notes

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A Tale of Two Futures: HIV and Antiretroviral Therapy in San Francisco

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The effect of antiretroviral therapy (ART) in preventing human immunodeficiency virus (HIV) infections and averting acquired immunodeficiency syndrome (AIDS) deaths in the San Francisco gay community over the next 10 years was predicted. A transmission model was coupled with a statistical approach that enabled inclusion of a high degree of uncertainty in the potential treatment effects of ART (in terms of infectivity and survival), increase in risky behavior, and rate of emergence of drug resistance. Increasing the usage of ART in San Francisco would decrease the AIDS death rate and could substantially reduce the incidence rate.

Currently, 30% of the San Francisco gay community are HIV-infected (*1*). About 50% of these HIV-infected men are taking combination ART (*2*); these three or more drug regimens include recently developed protease inhibitors, nonnucleoside reverse transcriptase inhibitors, or both. Part of the recent decrease in the San Francisco AIDS death rate (*3*) could be attributable to the effect of ART, as ART decreases disease progression rates (*4*). However, because treated individuals are likely to retain some degree of infectivity, it is possible that ART could lead to an increase in the infection rate (*5*). Furthermore, drug-resistant HIV strains (that are less responsive to therapy) have emerged (*6*), and risky behavior has begun to increase in San Francisco (*7*). Therefore, whether the epidemic-level effects of ART will be beneficial or detrimental is unclear.

To predict (with a degree of uncertainty) the effectiveness of ART in the San Francisco gay community, we developed and analyzed a mathematical model. Our model includes the potential effects of ART on the transmission dynamics of both drug-sensitive and drug-resistant HIV strains. It is specified by five ordinary differential equations (*8*) (Fig. 1) and allows for drug-resistant strains (that differ in their infectivity and disease progression rates from drug-sensitive strains) to emerge during treatment and to be sexually transmitted (*6*). Acquired resistance develops because of a variety of factors (*8*); we model the aggregate effect of all these factors by a single parameter r. We model the potential treatment effects of ART by assuming that ART [by reducing viral load (*9*)] increases average survival time and reduces infectivity, and that drug-resistant strains will be less responsive to therapy than drug-sensitive strains (*6*). Treatment (in our model) has three outcomes. A patient can respond to ART and remain as a nonprogressor for a specified amount of time, experience clinical failure and death without developing drug resistance (*9*), or virologically fail treatment and develop drug resistance (*10*). Individuals can go on and off ART, and drug-resistant infections can revert to drug-sensitive infections if the selective pressure of treatment is removed (*11*) (Fig. 1).

We predicted the effectiveness of a high usage of ART over the next 10 years in the San Francisco gay community by analyzing our model with time-dependent uncertainty analyses (*12*, *13*). Effectiveness was predicted in terms of the cumulative number of HIV infections prevented and the cumulative number of AIDS deaths averted (*14*). The San Francisco epidemic has been well studied, and the values of several of the parameters necessary for prediction are known (*15*); however, the values of other parameters are less certain. Hence, we conducted two uncertainty analyses (an optimistic and a pessimistic analysis) on the basis of different assumptions regarding the rate of increase in risky behavior and the rate of emergence of drug resistance. Both analyses included a high degree of uncertainty in the potential treatment effects of ART (on increasing survival and reducing infectivity). For the optimistic analysis we assumed that the rate of emergence of resistance would remain at a constant, fairly low value [only 10% of cases would acquire resistance per year (*16*)], and that risk behavior would not increase. For the pessimistic analysis we assumed that the rate of emergence of resistance could substantially increase [10 to 60% of cases could acquire resistance per year (*17*)], and that risk behavior could increase from almost no increase to a doubling (*17*).

For each uncertainty analysis we used our

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